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(Problem 2)

STUDY PLAN

B-VITAMIN CONTENT OF INNER BARK TISSUE OF LOBLOLLY PINE: EFFECT OF INFESTATION BY THE SOUTHERN PINE BEETLE AND ITS SYMBIOTIC MICROORGANISM COMPLEX

Prepared:

J. Robert Bridges — 9/15/77

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Research Entomologist
RWU-SO-2203

Date

Approved:

Peter L. Lorio, Jr. — 9/15/77

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Project Leader
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By

J. Robert Bridges

PROBLEM

The southern pine beetle (SPB), *Dendroctonus frontalis* Zimm., lives in association with many species of microorganisms (Barras and Perry, 1972; Moore, 1970; Howe *et al.*, 1971; Oberle, 1966; Keeth, 1968). SPB microbial associates include 2 species of specialized fungi which are propagated and transported in a specialized structure (mycangium) of the female beetle. This intimate association implies an obligate mutualistic relationship between the beetle and its mycangial fungi, and SPB growth and development are retarded in the absence of its fungi (Barras, 1973). A nutritional role for the fungi is possible but not enough is known about the SPB-microorganism complex to determine the specific benefits beetles derive from the microorganisms.

The beetle-microorganism complex increases the total nitrogen (N) concentration of loblolly pine inner bark (Hodges *et al.*, 1968). Growth of the beetle-microorganism complex also results in a decrease in the reducing sugar content of inner bark tissue (Barras and Hodges, 1969). These results suggest that the SPB-microorganism complex may benefit the beetle by increasing the normally low level of N in the inner bark or by decreasing the C : N ratio. However, more information is necessary before the role of the SPB symbionts can be understood. This study will investigate the influence of the beetle-microorganism complex on the vitamin content of loblolly pine inner bark.

Although a requirement by insects for the fat soluble vitamins A, D, E, and K has not been demonstrated, insects do require some of the B vitamins. Of the B vitamins, thiamine, nicotinic acid, pantothentic acid, pyridoxine, and riboflavin are probably required by all immature insects (House, 1965).

Because of the paucity of information on the vitamin content of tree bark, it is impossible to estimate whether enough of the B vitamins might be present in healthy bark to support SPB growth and development. One study by Wodsak and Ueckerman (1963) showed that pine bark contained small or undetectable amounts of B vitamins. Microorganisms, especially yeasts, are known sources of the B vitamins (Thompson, 1942; Harrow and Mazur, 1962) and could make a significant contribution to the B vitamin content of SPB food.

OBJECTIVE

The objective of this study is to compare the B vitamin content of healthy loblolly pine inner bark to bark that has been infected with SPB and its symbiotic microorganism complex to ascertain whether the symbiotic microorganisms provide the SPB with B vitamins.

METHODS

Healthy loblolly pine inner bark and bark infected by SPB-microorganism complex will be analyzed for the following B vitamins: thiamine, riboflavin, pyridoxine, pantothentic acid, and nicotinic acid. Infested bark will be obtained by inoculating loblolly pine bolts with SPB-microorganism complex or with pure cultures of the symbiotic fungi in the laboratory.

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Because of the paucity of information on the vitamin content of tree bark, it is impossible to estimate whether enough of the B vitamins might be present in healthy bark to support SPB growth and development. One study by Walsh and Beckerman (1963) showed that pine bark contained small or undetectable amounts of B vitamins. Microorganisms, especially yeasts, are known sources of the B vitamins (Thompson, 1942; Harrow and Mazur, 1962) and could make a significant contribution to the B vitamin content of SPB food.

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As necessary to obtain enough bark tissue for analysis, healthy loblolly pine trees will be felled and divided into 15 equal portions along the length of the boles. Five treatments, each replicated 3 times, will be randomly assigned to the bolts. The 5 treatments will be: 1) SPB-microorganism complex; 2) mycangial fungus, SJB 133; 3) mycangial fungus, SJB 122; 4) *Ceratocystis minor;* and, 5) uninoculated control.

Pure cultures of the fungi or pairs of beetles carrying its symbiotic complex will be introduced into the bolts through holes in the bark made with a sterile punch as previously described (Hodges *et al.*, 1968). Bolts will be stored in the laboratory at room temperature for 2-4 weeks to allow for growth of the microorganisms in bark tissue. Strips of inner bark approximately 1 cm wide will be removed from each side of the beetle galleries or adjacent to fungal inoculation points. The excised bark tissue will be lyophilized, and analyzed in duplicate for thiamine, riboflavin, pantothentic acid, nicotinic acid, and pyridoxine.

For vitamin analyses, the chemical and microbiological methods of the Association of Offician Analytical Chemists (1970), the Association of Vitamin Chemists (1966), Strohecker and Henning (1965), Gyorgy and Pearson (1967), will be adapted as necessary and used for analysis of bark samples.

Data for B vitamin concentration of bark tissues will be analyzed using an analysis of variance as summarized in the following:

Source of Variation	df
Treatment	4
Replication within treatment	10
Sampling error	15
Total	29

Treatment means will be compared using Duncan's New Multiple Range Test.

As necessary to obtain enough bark tissue for analysis, healthy looking pine trees will be felled and divided into 15 equal portions along the length of the boles. Five treatments, each replicated 3 times, will be randomly assigned to the bolts. The 5 treatments will be: 1) SFB-microorganism complex; 2) mycangial fungus, SLB 133; 3) mycangial fungus, SLB 122; 4) Cerotocystis minor; and, 5) uninoculated control.

Pure cultures of the fungi or pairs of beetles carrying its symbiotic complex will be introduced into the bolts through holes in the bark made with a sterile punch as previously described (Hodges et al., 1968). Bolts will be stored in the laboratory at room temperature for 2-4 weeks to allow for growth of the microorganisms in bark tissue. Strips of inner bark approximately 1 cm wide will be removed from each side of the beetle galleries or adjacent to fungal inoculation points. The excised bark tissue will be lyophilized and analyzed in duplicate for thiamine, riboflavin, pantothenic acid, nicotinic acid, and pyridoxine.

For vitamin analyses, the chemical and microbiological methods of the Association of Official Analytical Chemists (1970), the Association of Vitamin Chemists (1965), Stroneker and Henning (1965), Cooney and Pearson (1957), will be adapted as necessary and used for analysis of bark samples.

Data for B vitamin concentration of bark tissues will be analyzed using an analysis of variance as summarized in the following:

Source of Variation	df
Treatment	4
Replication within treatment	10
Sampling error	15
Total	29

Treatment means will be compared using Duncan's New Multiple Range Test.

PRESENTATION OF RESULTS

A final report and manuscript(s) as warranted will be prepared upon completion of the study.

DATES OF ESTABLISHMENT AND COMPLETION

Literature review and preliminary work on methods of vitamin analyses were begun in January, 1977. The study will be completed by December, 1978.

PERSONNEL ASSIGNMENT

J. Robert Bridges	-	Research Entomologist
Faye H. Guinn	-	Biological Lab. Tech.
Thelma Perry	-	Biological Lab. Tech.

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FS-SO-2203-1.39 "B-vitamin content of inner bark tissue of loblolly pine: effect of infestation by the southern pine beetle and its symbiotic ~~microorganism complex.~~

(STUDY NUMBER) (TITLE)

X Robert Bridges 8/11/77

(AUTHOR SIGNATURE) (DATE)

J. R. Bridges 8/11/77

REMARKS:

SUBMITTED: Peter P. Lorio, Jr. 8-11-77

(PROJECT LEADER SIGNATURE) (DATE)

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INSTRUCTIONS

1. Project Leader must submit this form with each study plan. Also use this form when manuscripts are submitted for review.
2. Insert desired "Routing" in space at top of form.
3. Be sure to insert proper identification in the "Research Work Unit" block.
4. Completed copies of this form will be filed in the study file in the Assistant Director's and Project Leader's files.

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